

Figure 1

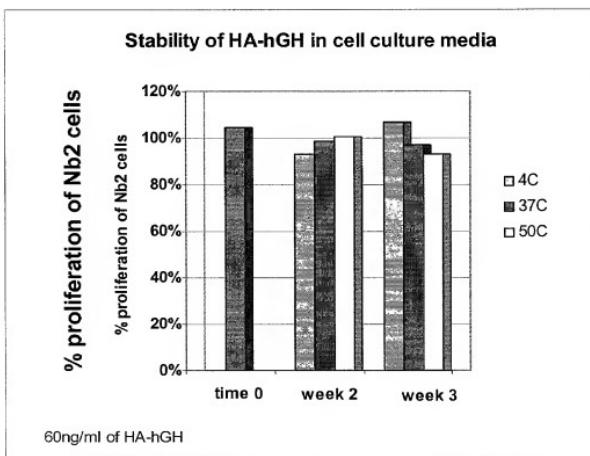


Figure 2

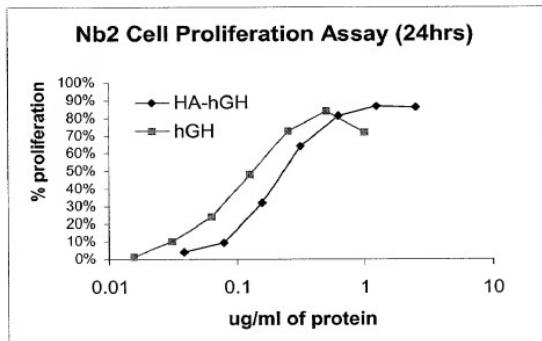


Figure 3A

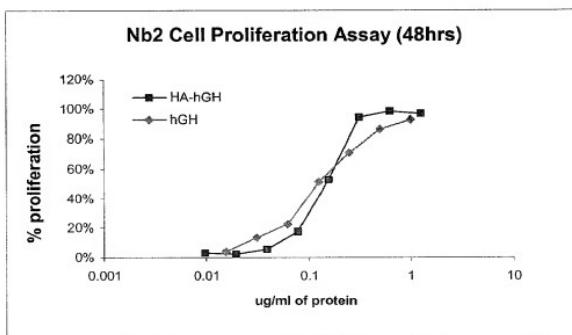
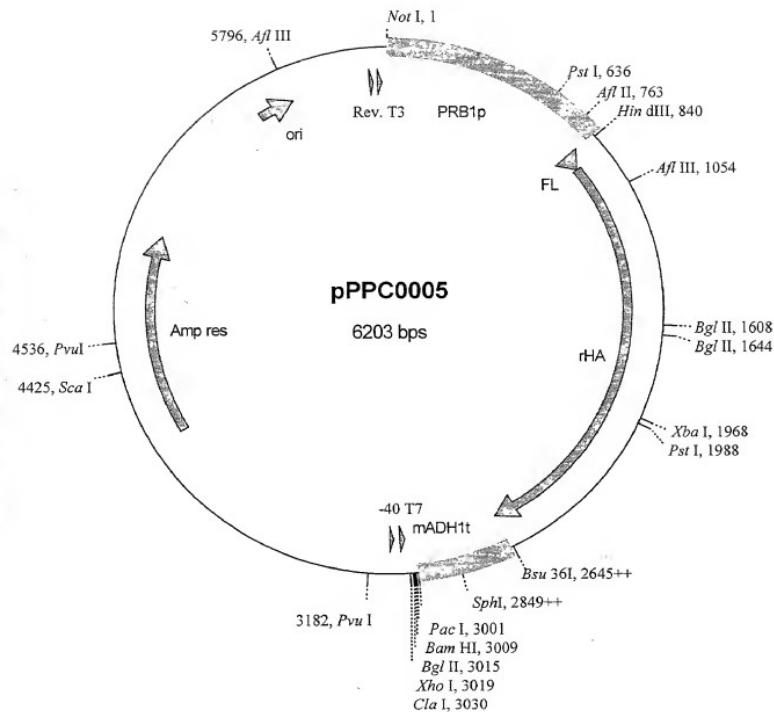


Figure 3B

TOEPLITZ-STEES60

**Figure 4**

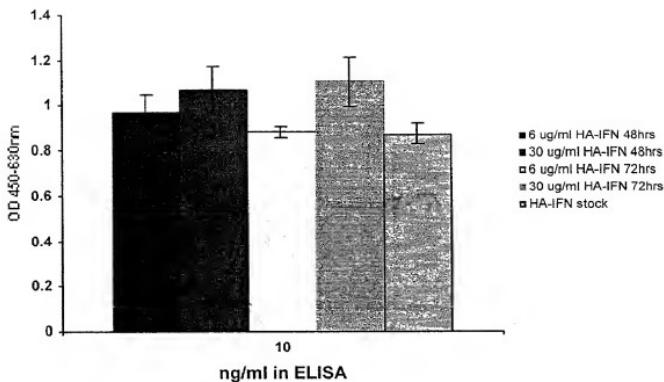


Figure 5

Figure 6

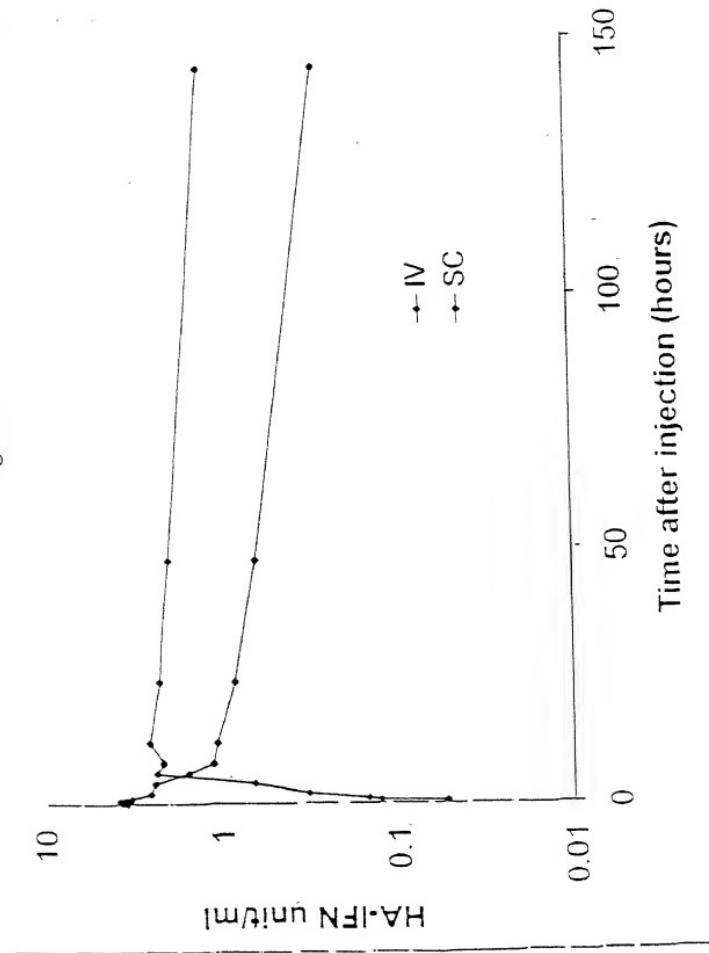
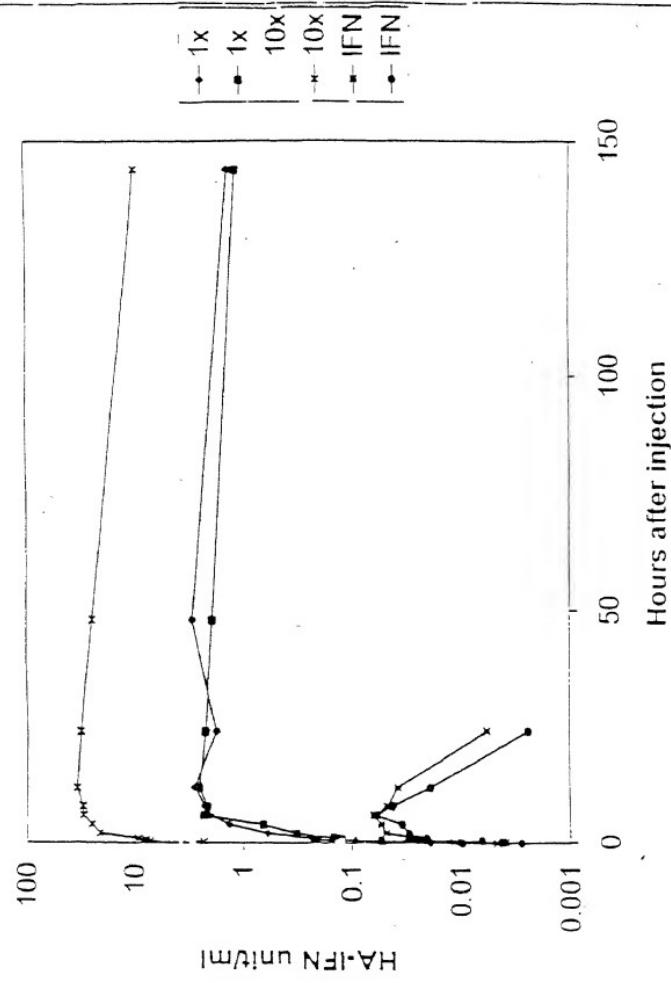


Figure 7



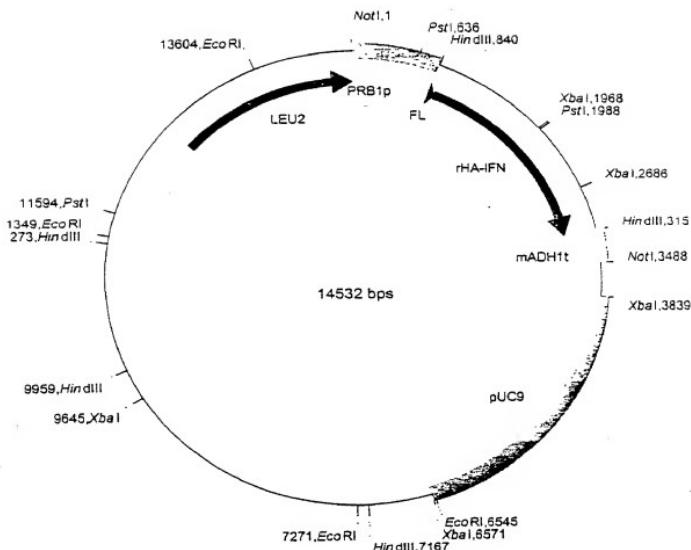


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises
PRB1 promoter, from *S. cerevisiae*.
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of
 the MIF α -1 leader.
 HA-IFN α coding sequence with a double stop codon (TAATAA)
ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence
 normally present in the Hind III/Bam HI fragment generally used.

Figure 8

Localisation of ‘Loops’ based on the HA Crystal Structure which could be used for Mutation/Insertion

Loop	Loop
I Val54-Asn61	VII Glu280-His288
II Thr76-Asp89	VIII Ala362-Glu368
III Ala92-Glu100	IX Lys439-Pro447
IV Gln170-Ala176	X Val462-Lys475
V His247-Glu252	XI Thr478-Pro484
VI Glu266-Glu277	XII Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

IV

151	APELLFFAKR YKAAFTECC <u>Q</u> <u>AADKAACLLP</u> KLDDELRLDEGK ASSAKQRLKC HHHHHHHHHHHH HHHHHHHHHH HHHHHH HHHHHHHHHHHH HHHHHHHHHHHH
151	APELLFFAKR YKAAFTECC <u>X</u> <u>XXXXXXCLLP</u> KLDDELRLDEGK ASSAKQRLKC HHHHHHHHHHHH HHHHHHHHHH HHHHHH HHHHHHHHHHHH HHHHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

$(X)_n$
 ↓
 IV

151	APELLFFAKR YKAAFTECC <u>Q</u> <u>AADKAACLLP</u> KLDDELRLDEGK ASSAKQRLKC HHHHHHHHHHHH HHHHHHHHHH HHHHHH HHHHHHHHHHHH HHHHHHHHHHHH
-----	--

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

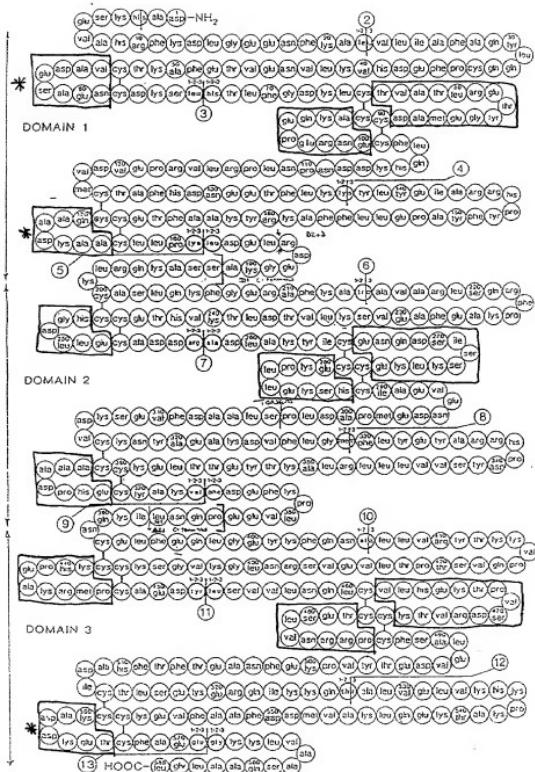
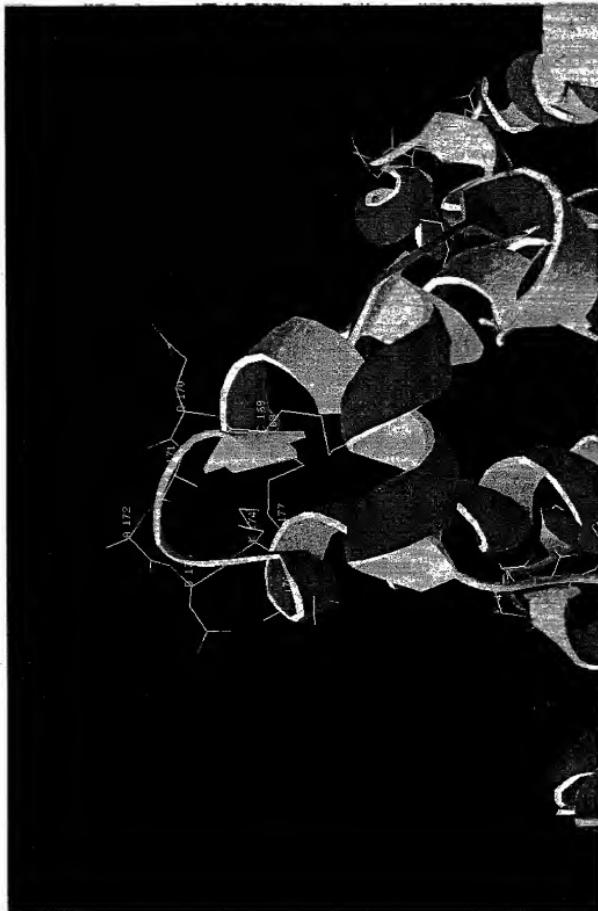


Figure 11



Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176

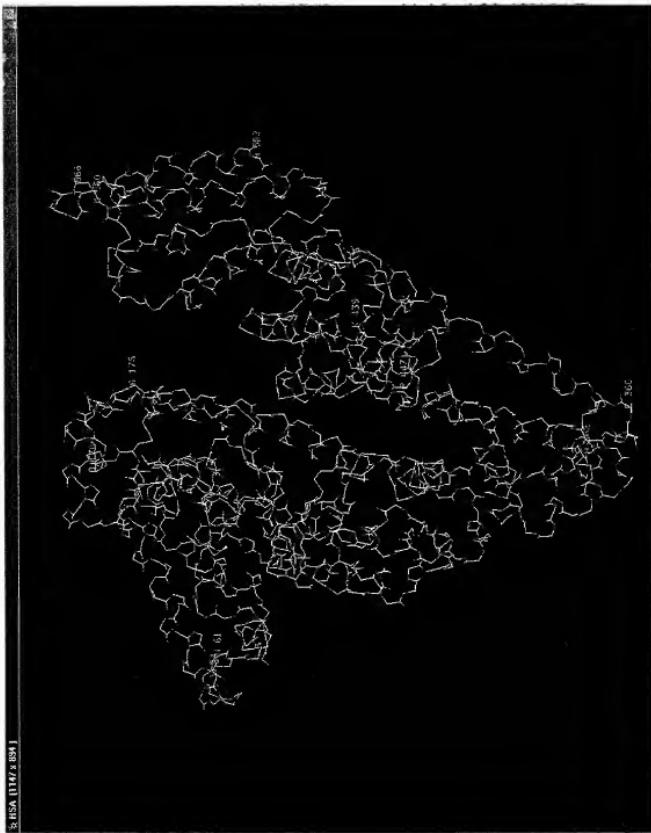
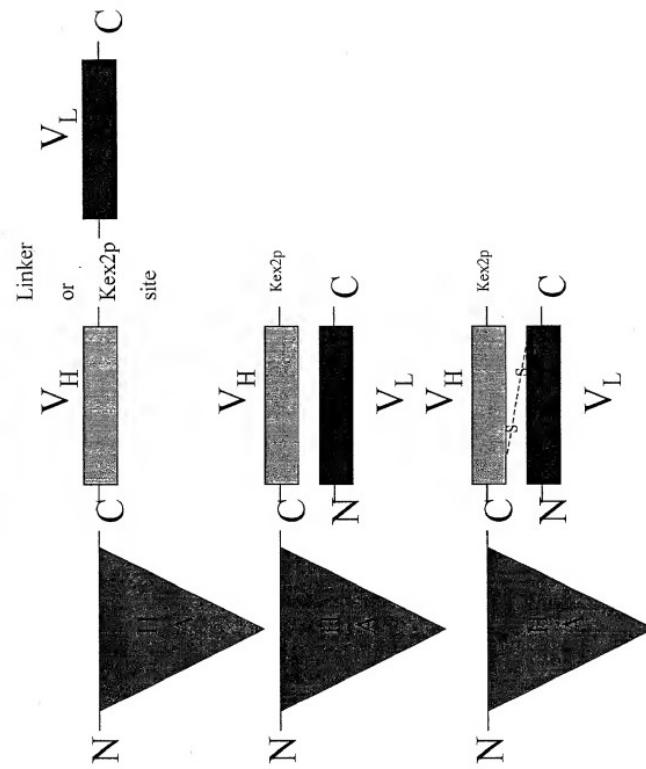


Figure 13: Tertiary Structure of HA



**Figure 14: Schematic Diagram of Possible ScFv Fusions
(Example is of a C-terminal fusion to HA)**

TRANSCRIPTION

15/18

1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAA 60
1 D A H K S E V A H R F K D L G E E N P K 20

61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GAA GAA ACT GAA TTT GCA AAA ACA TGT GCT GAT GAG TCA GCT GAA 180
41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GAT GCA ACT CTT 240
61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA GAA CCT GAG AGA AAT GAA 300
81 R E T Y G B M A D C C A K Q E P E R N E 100

301 TGC TTC TTG GAA GAC AAC AAC AAC CTC CCC CGA TTG GTG AGA CCA GAG CCT GTT 360
101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT GCT TTT GAT GAC AAT GAA GAG AGA TTT TTG AAA AAA TAC TTA TAT 420
121 D V M C T A F H D N E E T P L K K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTT GCT AAA AGG 480
141 E I A R R H P Y F Y A P E L L F F A K R 160

Figure 15A

T G C T T G T G T G A T T G C C G G G

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481 TAT AAA GCT GCT TTT AGA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCT GTC CTC CTG TTG CCA 540
161 Y K A A F T B C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AGG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC GAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A S L Q K P F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTC TCC AAC TTA GTG AGA GAT CTT ACC AAA 720
221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CCT 780
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AGG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC ACT AAA CTG AAG GAA TGC TGT GAA 840
261 A K Y I C E N Q D S I S S K L K E C C E 280

841 AAA CCT CTG TTG GAA AAA TCC GCA ATT GCC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT CCT 900
281 K P L L E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTC GAA AGT AAG GAT GTT TGC AAA AAC TAT CCT 960
301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B

TRANSLATION STUFF

17/18

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TGT TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GNG CTG CTG CTG AGA CTT GCC ACG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
361 Y S V V L L R L A R T Y E T T L E K C 360

1081 TGT GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GNG TGC GAT GAA TTT AAA CCT CTT 1140
361 C A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT GAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GGA GAG 1200
381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG IAA GTA CCC CAA GTG TCA ACT 1260
401 Y K F Q N A L L V R Y T K V P Q V S T 420

1261 CCA ACT CTT GTC GAG GTC TCA AGA AAC CTA GAA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GTC GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTC AAC CAG TTA 1380
441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GNG TTG CAT GAG AAA AGC COA GTC AGT GAC AGA GTC AGA AAA TGC TGC ACA GAG TCC 1440
461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

FOUNDATIONS OF GENETICS

18/18

1441 TPG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GCA TAC GAT GAA CCC GTT CCC AAA 1500
481 L V N R R P C F S A L E V D E T Y V P K 500

1501 GRG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
501 E F N A E T F T F H A D I C T L S E K P K E 520

521 R Q I K K Q T A L V E L V E L V K H K P K A T 540

1561 AGA CAA ATC ATG AAA CAA ACT GCA CTT GAG CTT GTC AAA CAC AAG CCC AAG GCA ACA 1620
541 K E Q L K A V M D D P A A F V B K C C K 560

1621 AAA GAG CAA CTG AAA GCT GTC GAT GAT GAT GTC GCA GCT TTT GTC GAG GAG TGC TGC AAG 1680
561 A D K E T C F A E B G K K L V A A S Q 580

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
581 A L G L * 585

Figure 15D